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Date: September 8, 2004


Paige A. Johnson

Attorney Docket No. 11000.1037c5
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of **Matthew SLEEMAN and J. Greg MURISON**

Group Art Unit: 1614

Serial No. : 10/613,413
Filed : July 3, 2003
For : **FIBROBLAST GROWTH FACTOR RECEPTORS
AND METHODS FOR THEIR USE**
Examiner : Ruixiang Li

DECLARATION OF DR. J. GREG MURISON

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

The undersigned, Dr. J. Greg Murison, hereby declares:

1. I am presently Head of Research and Development at Genesis Research and Development Corporation Limited, the assignee of the subject patent application, and an inventor of the claimed subject matter. I have a PhD in the field of Immunology. The following studies were carried out under my supervision.

2. The effects of administration of FGFR5 protein *in vivo* were examined as follows:

Experiments 1 and 2

Experiment 1 used BALB/cByJ mice and experiment 2 used C3H/HeJ mice. Both sets of mice were injected subcutaneously with 5 μ g (55 nM in 0.1 ml PBS) of murine FGFR5 β

extracellular domain (ECD; amino acids 22 - 373 of SEQ ID NO: 6) - murine IgG3 Fc fusion protein (prepared as described in Example 5 of the instant specification) in the morning and the same dose in the evening (i.e. each mouse received 10 µg per day) for five days. Control mice received PBS alone. On the sixth day, the mice were sacrificed and the draining lymph nodes (axillary and lateral axillary) were removed. A single cell suspension was generated from the lymph nodes of each mouse and the number of cells collected from each mouse was determined by trypan blue viability counting using a haemocytometer. The lymph node cells collected from the FGFR5-treated mice were then pooled. The lymph node cells collected from the PBS-treated mice were amalgamated into a separate pool of cells. The cells from both the FGFR5 and PBS-treated mice were then stained for the cell surface antigens listed in Table 1, below, and analyzed by flow cytometry. The data provided in Table 1 is expressed as a percentage of the lymph node cells that express the marker examined.

Experiment 3

In this experiment, C3H/HeJ mice were injected subcutaneously with 10 µg (110 nM in 0.1 ml PBS) of murine FGFR5 β ECD - human IgG1 Fc fusion protein in one injection per day for 5 days. While the treatment regime differed from that used in Experiments 1 and 2 above, the total dose of protein administered to the mice did not alter. Control mice were administered human IgG1 Fc fragments alone. On the sixth day, the mice were sacrificed and the draining lymph nodes (axillary and lateral axillary) removed. The number of cells collected from each mouse and the presence of cell surface antigens was determined as described above.

In all three of these experiments, *in vivo* administration of FGFR5 stimulated lymphadenopathy, or enlargement of the lymph nodes. More specifically, administration of FGFR5 was found to result in a preferential increase in the frequency of B cells in the draining lymph nodes. When compared to mice treated with Fc protein, the frequency of B cells doubled in the draining lymph nodes of FGFR5-treated mice. An analysis of the cell cycle state of the B cells by flow cytometry indicated that they were not expanding but were either selectively migrating or being retained in the lymph nodes. The cells are, however, activated as there is an increase in the number of cells expressing the very early activation antigen, CD69. Similar results were

obtained for both the murine FGFR5 β ECD - human IgG1 Fc fusion protein and the murine FGFR5 β ECD - murine IgG3 Fc fusion protein, demonstrating that the effects are caused by the segment common to both fusion proteins, i.e. the murine FGFR5 β extracellular domain.

Table 1: Comparison of three *in vivo* experiments testing the effects of *in vivo* administration of soluble FGFR5 in mice

(The values in this table represent the percentage of total lymph node cells expressing the indicated marker)

		<u>Experiment 1</u>		<u>Experiment 2</u>		<u>Experiment 3</u>	
		<u>Balb/c</u>		<u>C3H/HeJ</u>		<u>C3H/HeJ</u>	
Markers	Cell type recognized	<u>Murine Fc FGFR5</u>	<u>PBS</u>	<u>Murine Fc FGFR5</u>	<u>PBS</u>	<u>Human Fc FGFR5</u>	<u>Human Fc</u>
CD3	T cell	63	81	59	82	32	67
I.CD19	II.B cell	35	21	39	16	61	26
Class II	B cell and macrophage	41	20	ND*	ND	ND	ND
CD45R	B cell	ND	ND	ND	ND	72	31
CD69	Activated cells	23	14	18	10	21	10

* ND = Not determined

Experiment 4

The popliteal lymph node assay was used to assess the effects of treating mice with a murine FGFR5 γ -Fc fusion protein. The polypeptide sequence for FGFR5 γ is provided in SEQ ID NO: 7. Groups of 4 BALB/cByJ mice were injected with 50 μ g of FGFR5 γ -Fc under the left hind footpad and 50 μ g of the control protein FGFR2-Fc under the right hind footpad. In addition, groups of two mice were injected with PBS under the left hind footpad to compare the effects of FGFR5, FGFR2 and PBS. The lymphatics from this site drain to the popliteal lymph node. These lymph nodes were collected 1, 2 and 3 days after the initiation of the experiment and the cells from each node were released and counted using a haemocytometer, and their viability assessed by the Trypan blue exclusion assay. The cells from the individual nodes were then

stained with fluorescently labeled antibodies and the relative frequencies of each of the major haemopoietic cell types assessed by flow cytometry.

The results of these assays are shown in Figs. 1-5 submitted herewith. Specifically, Fig. 1 shows that subcutaneous administration of FGFR5 γ -Fc was found to induce a localized lymphadenopathy in the draining popliteal lymph nodes. More specifically, FGFR5 γ -Fc induced an increase in the total number of cells isolated from the popliteal lymph nodes that was apparent 24 hrs after the protein had been administered and rose to 3.2 times the number of cells isolated from the nodes draining the FGFR2 injection site. The data provided in Fig. 2 demonstrates that subcutaneous administration of FGFR5 γ -Fc induced a statistically significant increase in the numbers of B cells (CD19+) and activated B cells (CD19+CD69+) 2 and 3 days after treatment with FGFR5 γ -Fc and FGFR2-Fc fusion proteins. Fig. 3 shows that subcutaneous administration of FGFR5 γ -Fc induced a statistically significant increase in the frequency of B cells (CD19+) and activated B cells (CD19+CD69+) 2 and 3 days after treatment with the FGFR5 γ and FGFR2-Fc fusion proteins. Fig. 4 shows that subcutaneous administration of FGFR5 γ -Fc induced a statistically significant increase in the numbers of T cells (CD3+) and activated T cells (CD3+CD69+) 3 days after treatment with the FGFR5 γ and FGFR2-Fc fusion proteins. Fig. 5 shows that subcutaneous administration of FGFR5 γ -Fc induced a decrease in the frequency of T cells (CD3+) 2 days after treatment and activated T cells (CD3+CD69+) 3 days after treatment with the FGFR5 γ and FGFR2-Fc fusion proteins. In Figs. 1-5, the columns marked with an asterisk denote an FGFR5 γ -Fc treatment group that differs significantly ($p < 0.05$) from the FGFR2-Fc controls as assessed by the student's T test.

These experiments demonstrate that FGFR5 induced a localized B cell dominated lymphadenopathy, as shown by an increase in the total number of cells extracted from the lymph node and a preferential increase in both the number and percentage of activated B cells (CD19+CD69+ cells). All of the FGFR5 induced changes were most apparent 3 days after treatment. Although the frequency of T cells declined in the lymph nodes collected from the FGFR5 treated mice, the absolute number of T cells per node increased. These data show that FGFR5 activates the immune system and therefore has the ability to augment responses to antigens in an adjuvant-like manner.

3. An alignment of the extracellular domain (ECD) of the murine FGFR5 β sequence of SEQ ID NO: 6 with the sequence of the ECD of human FGFR5 provided in Fig. 10 of the specification as originally filed (corrected SEQ ID NO: 8) is attached hereto as Exhibit A, with an alignment of the ECD of the murine FGFR5 γ sequence of SEQ ID NO: 7 with the human FGFR5 ECD sequence of corrected SEQ ID NO: 8 being provided in Exhibit B. Based on these alignments, I would expect the polypeptide shown in Fig. 10 (corrected SEQ ID NO: 8) to have essentially the same functional properties as the polypeptides of SEQ ID NO: 6 and SEQ ID NO: 7 employed in the above experiments.

4. The undersigned further declares that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 35 of the United States Code.



J. Greg Murison, Ph.D.

31 August 2004
Date



Figure 1:

Average number of cells per 1 Lymph node from mice treated with PBS or FGFR2Fc or FGFR5Fc on Day +1, +2 and +3 after treatment

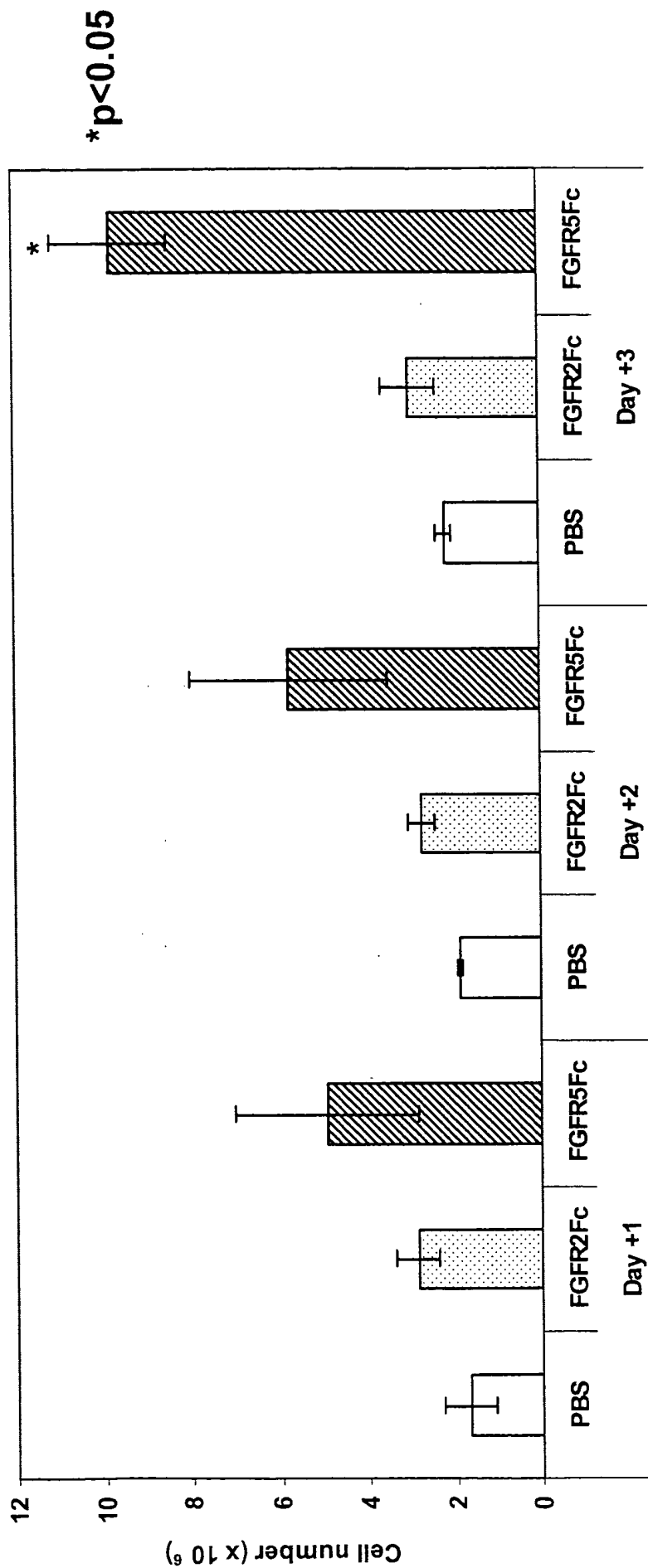


Figure 2:

Number of B cells in individual lymph nodes from mice treated with FGFR5Fc or controls by subcutaneous footpad-injections on Days +1, +2 and +3 after treatment.

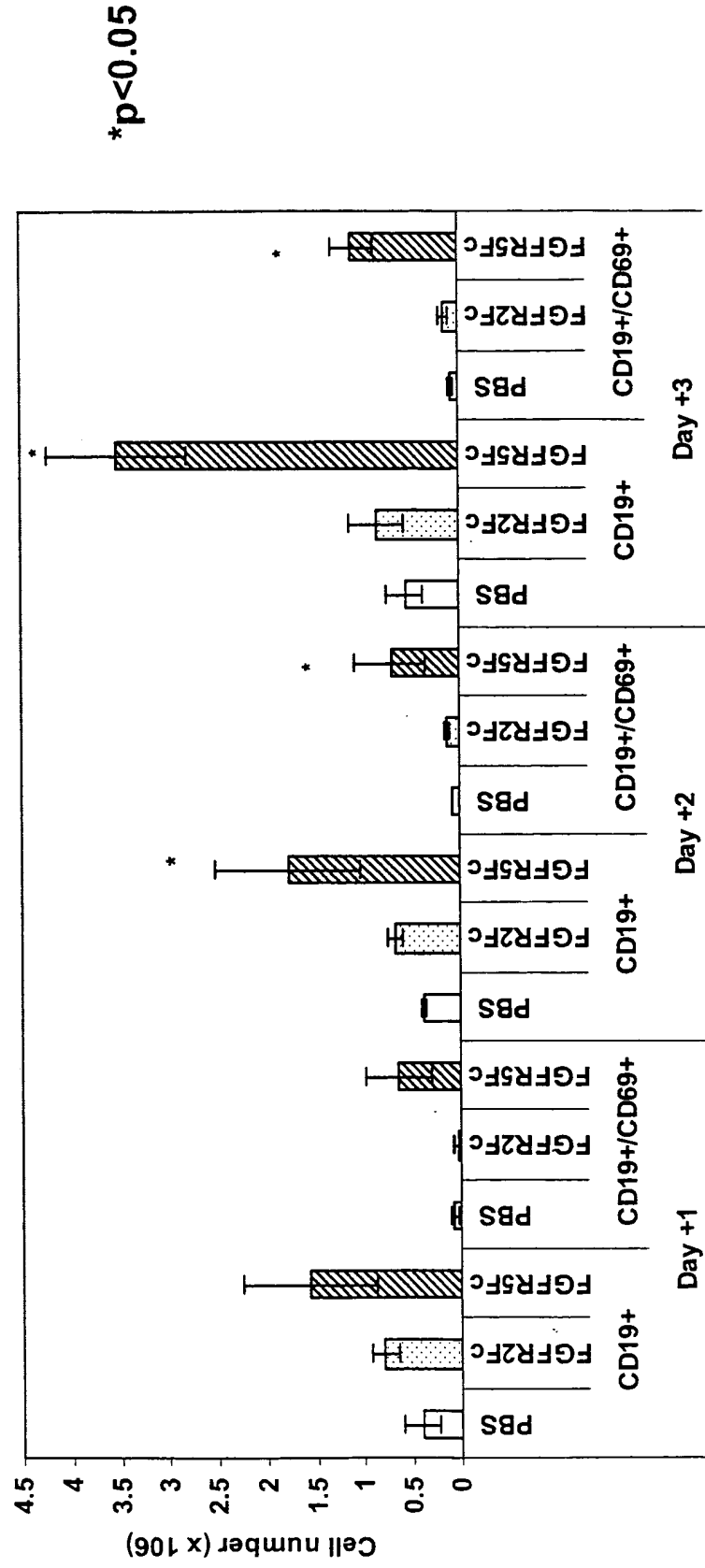


Figure 3:

Frequency of B cells in individual lymph nodes from mice treated with FGFR5Fc or controls by subcutaneous footpad-injections on Days +1, +2 and +3 after treatment.

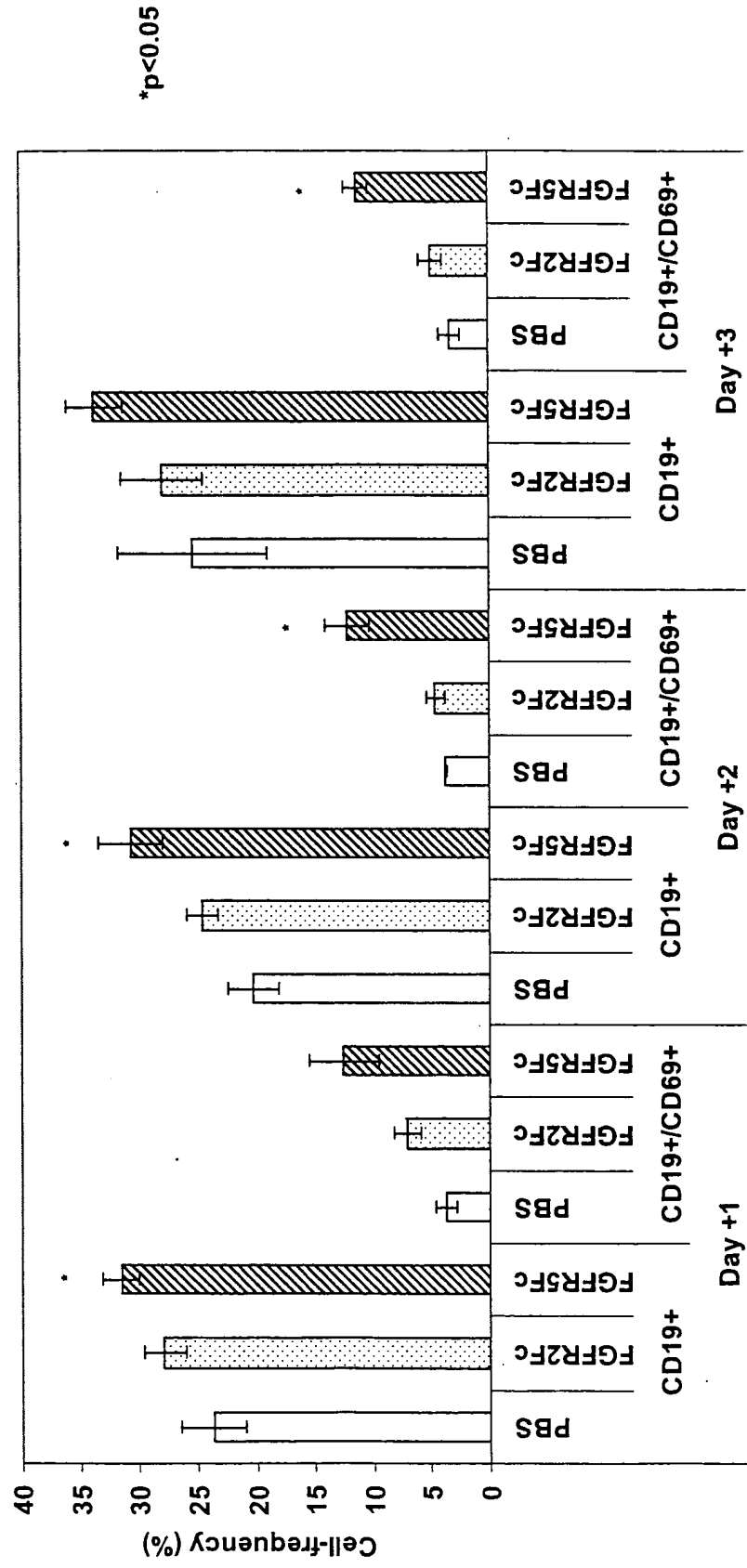


Figure 4:

Number of T cells in individual lymph nodes from mice treated with FGFR5Fc or controls by subcutaneous footpad-injections on Days +1, +2 and +3 after treatment.

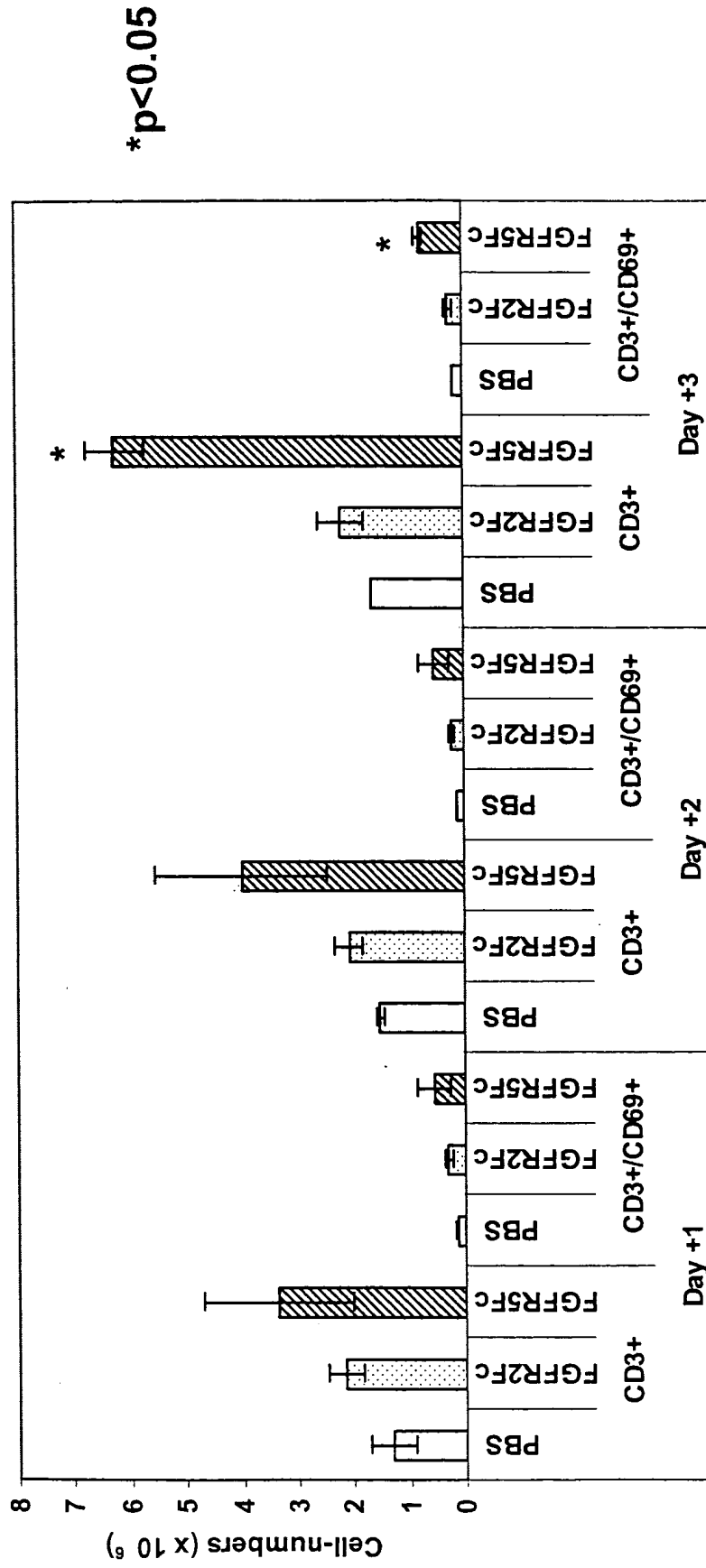


Figure 5:

Frequency of T cells in individual lymph nodes from mice treated with FGFR5Fc or controls by subcutaneous footpad-injections on Days +1, +2 and +3 after treatment.

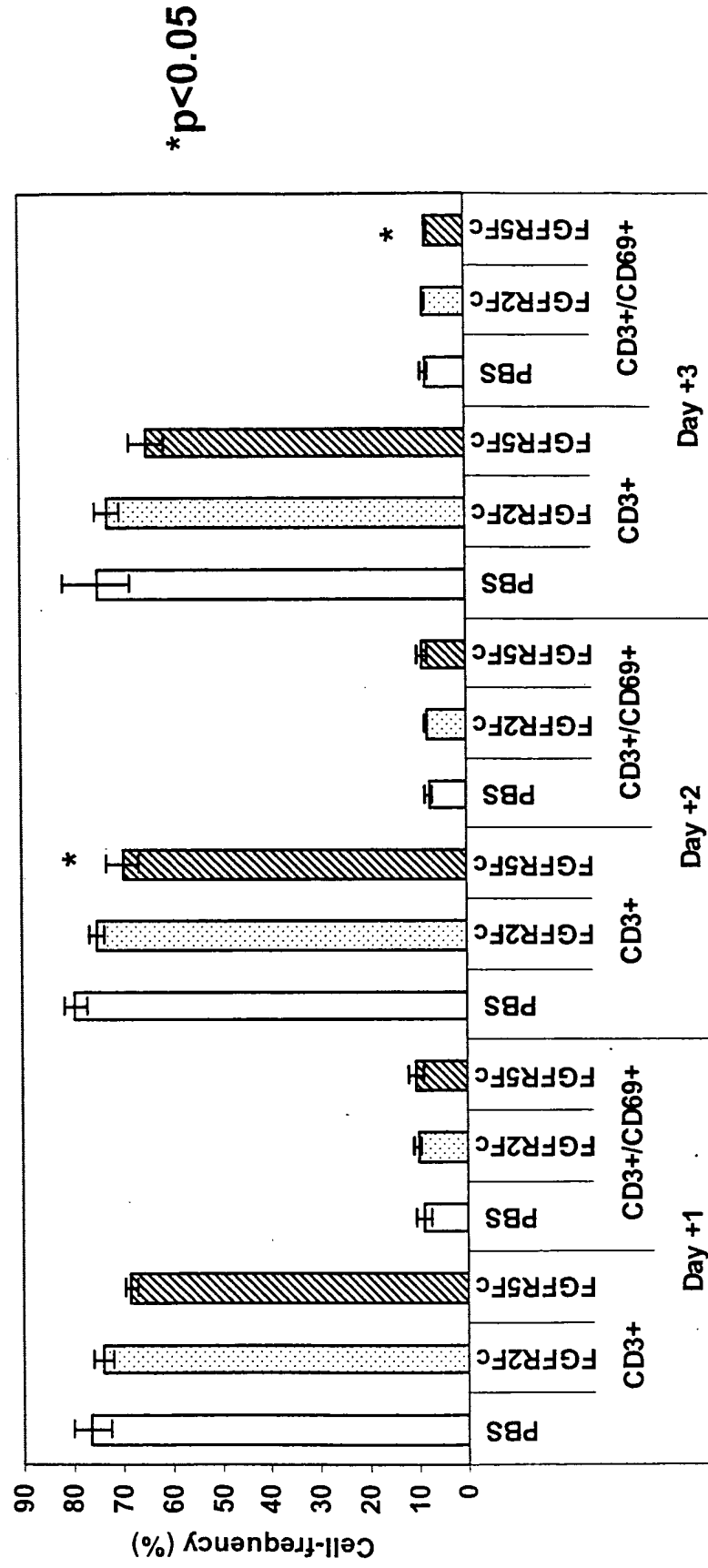




EXHIBIT A

Alignment of muFGFR5 β (SEQ ID NO: 6) and huFGFR5 (SEQ ID NO: 8):

Blue highlighting: three Ig domains
 Crimson highlighting: Acidic Box
 Underlined: CAM/heparin binding domains
 White in blue highlights: Conserved cysteines
 Yellow highlighting: Transmembrane domain

The alignment is:

CLUSTAL W (1.75) multiple sequence alignment

```

muFGFR5 $\beta$       -----MTRSPALLLLL---LGALPSAEAARGP---PRMAD-----KVVPRQVARL
huFGFR5        -----MTPSPLLLLLLPPLLLGAFPPAAAARGP---PKMAD-----KVVPRQVARL
                  :           .           *           *           :

fgFR5 $\beta$          [ ] C [ ]
huFGFR5         [ ] C [ ]
                  * *           * : *           *           : * * * *

fgFR5 $\beta$          SLSVNYTLIIMDDISPG [ ] ---GSSGGQED-PASQQWARPRFTQPSKMRRRVIAR
huFGFR5         SLSVNYTLVVLDDISPG [ ] ---SSSGGQED-PASQQWARPRFTQPSKMRRRVIAR
                  : . . . . . : .           * : * . . : * : *

fgFR5 $\beta$          P [ ] C [ ]
huFGFR5         P [ ] C [ ]
                  * . . . : * : * * . : * : : : : : . . . * : : : * * * *

fgFR5 $\beta$          [ ] C [ ] INATYKVDVIQTRSKPVLTGTHPVNTTVDF [ ] C [ ]
huFGFR5         [ ] C [ ] INATYKVDVIQTRSKPVLTGTHPVNTTVDF [ ] C [ ]
                  ** * * * * : * . * : * : * : * * . * * . . : * * * * : * *

fgFR5 $\beta$          [ ]
huFGFR5         [ ]
                  * : * : : . . . . . : * : . : : : :

fgFR5 $\beta$          [ ] YSFRSAFLTVPDPKPPGPPMASSSSSTSLPWPVVIGIPAGAVFILGTVLLWLC
huFGFR5         [ ] YSFRSAFLTVPDPKPPGPPMASSSSSTSLPWPVVIGIPAGAVFILGTVLLWLC
                  -----

fgFR5 $\beta$          QTKKK-----PC
huFGFR5         -----PC
                  -----

fgFR5 $\beta$          APASTLPVPG-----HRPPGTSRERSGDKDLPSLAVGICEEHGSAMAPQHILASGST
huFGFR5         -----

fgFR5 $\beta$          AGPKLYPKLYTDVHTHTHT--HTCTHTLSCGGQGSSTPACPLSVLNTANLQALCPEVGIW
huFGFR5         -----

fgFR5 $\beta$          GPRQQVGRIENNGGRVS-----
huFGFR5         -----
  
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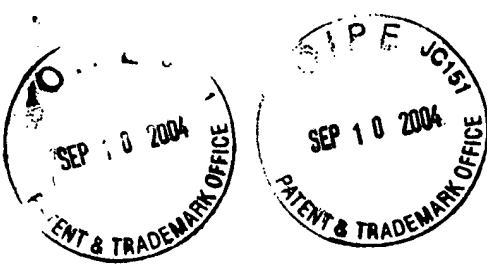


EXHIBIT B

Alignment of muFGFR5y (SEQ ID NO: 7) and huFGFR5 (SEQ ID NO: 8):

Blue highlighting: two Ig domains
Underlined: CAM/heparin binding domains
White in blue highlights: Conserved cysteines
Yellow highlighting: Transmembrane domain

The alignment is:

CLUSTAL W (1.75) multiple sequence alignment

```
muFGFR5y      MTRSPALLLLL----LGALPSAEAAR-----
huFGFR5       MTPSPLLLLLLPPLLLGAFPPAAAARGPPKMADKVVPRQVARLGRTVRLQCPVEGDPPPL
               ** ** *****      ***:* * **

muFGFR5y      -----DDI
huFGFR5       TMWTKDGRTIHSGWSRFRVLPQGLKVKQVEREDAGVYVCKATNGFGSLSVNYTLVVLLDDI
               ***

muFGFR5y      SPGKESPGPGSSGGQEDPASQQWARPRFTQPSKMRRRVIA[P]C[P]
huFGFR5       SPGKESLGPDSGGQEDPASQQWARPRFTQPSKMRRRVIA[P]C[P]
               ***** **..*****

muFGFR5y      [P]C[P]INATYKVDVIQ
huFGFR5       [P]C[P]INATYKVDVIQ
               ** *****:* *: * *****:*****:*****

muFGFR5y      RTRSKPVLTGTHPVNTTVDF[P]C[P]
huFGFR5       RTRSKPVLTGTHPVNTTVDF[P]C[P]
               *****:*****

muFGFR5y      [P]C[P]YSFRSAFLTVPDPK
huFGFR5       [P]C[P]YSFRSAFLTVPDPK
               ***** *

muFGFR5y      PPPGPPMASSSSSTSLPWPVVGIPAGAVFILGTVLLWLCQTKKKPCAPASTLPVPGHRP
huFGFR5       PPPGPPMASSSSSTSLPWPVVGIPAGAVFILGTVLLWLCQTKKKPCAPASTLPVPGHRP
               -----

muFGFR5y      PGTSRERSGDKDLPSLAVGICEEHGSAMAPQHILASGSTAGPKLYPKLYTDVHTHTHTHT
huFGFR5       PGTSRERSGDKDLPSLAVGICEEHGSAMAPQHILASGSTAGPKLYPKLYTDVHTHTHTHT
               -----

muFGFR5y      CHTLSCGGQGSSTPACPLSVLNTANLQALCPEVGIWGPRQQVGRIENNGGRVS
huFGFR5       CHTLSCGGQGSSTPACPLSVLNTANLQALCPEVGIWGPRQQVGRIENNGGRVS
               -----
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